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The Anti-inflammatory Activity of Metal Complexes and Salts of Amine Carboxyboranes

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The metal complexes and salts of amine carboxyboranes were shown to be potent anti-inflammatory agents in rodents at 8 mg kg^{-1}. They were effective in blocking induced edema, pleurisy and endotoxic shock while blocking both local and central pain processes. The ability of the agents to function as anti-inflammatory agents is multi-fold. First, lysosomal enzymes of specific cells, e.g. macrophages, were inhibited with IC_{50} values in the range of 10^{-6} M. Collagenase I and II activities were inhibited with IC_{50} values approximately equal to 10^{-4} M. The anti-inflammatory activity of these agents at the molecular levels appears to be due to inhibition of the release of TNFα and Il-1 from macrophages which indirectly control chemotaxic migration of white blood cells as indicated by the suppression of PMN and macrophage invasion into sponges implanted subcutaneously (SC) in mice. Furthermore, in these invading cells, the agents' blockage of TNFα and Il-1 or Il-2 release down-regulates prostaglandin and leukotriene enzymatic synthetic rates and, consequently, their release, resulting in a reduction of the inflammation process.

Keywords: Inflammation, amine carboxyboranes, metal complexes, HCT-8 toxicity

INTRODUCTION

The amine carboxy- and cyanoboranes have been reported previously to inhibit induced inflammation in rodents.[1] The agents blocked the release of lysosomal enzymes in mouse liver and polymorphonuclear neutrophils (PMNs). Oxidative phosphorylation of PMNs was uncoupled and prostaglandin synthetase activity in beef seminal vesicles was inhibited by the agents.[1] The present study concentrated on metal complexes and salts of amine carboxyboranes so as to examine in depth their mode of action as anti-inflammatory agents.

MATERIALS AND METHODS

Source of compounds. Na_2BH_3COO (**1**), $Na(CH_3)_3NBH_2COO \cdot 0.25CH_3OH$ (**2**),[2] $Na(CH_3)_2$ $NHBH_2COO \cdot 0.45H_2O$ (**3**),[2] $[Fe_3O((CH_3)_3NBH_2COO)_6(CH_3OH)_3]NO_3 \cdot CH_3CN$ (**4**), $[Fe_3O((CH_3)_3NBH_2COO)_6(CH_3OH)_3]Cl$ (**5**),[3] $[Cr_3O((CH_3)_3NBH_2COO)_6(H_2O)_3] \cdot NO_3 \cdot CH_3OH \cdot CH_3CN$ (**6**),[3] *cis*-$[Co(en)_2((CH_3)_3NBH_2COO)_2]Cl \cdot 2.5\,H_2O \cdot 0.5\,CH_3OH$ (**7**),[3] $Ca((CH_3)_3NBH_2COO)NO_3 \cdot CH_3COCH_3 \cdot 0.5H_2O$ (**8**),[3] $Co((CH_3)_3NBH_2COO)NO_3$ CH_3CNCH_3OH (**9**)[3] and $[Cu_2(Me_3NBH_2COO)_4 \cdot 2(Me_3NBH_2COOH)]$ (**10**))[4] were all previously synthesized by literature methods. All chemicals were purchased from Aldrich Chemical Co., (Milwaukee, WI). Radioisotopes were obtained from New England Nuclear (Dupont, Boston, MA) and substrates and cofactors were obtained from Sigma Chemical Company (St Louis, MO).

in vivo tests

Anti-inflammatory screen in mice and rats. Male CF_1 mice weighing 27.5–32 g obtained from Jackson Lab. (Bar Harbor, MA) were used to screen agents at 8 mg kg^{-1} intraperitoneal [IP] × 2 administered 3 h and 30 min prior to administering the irritant, according to Winter's protocol.[5] Evaluation of the induced edema was made by injecting 2% carrageenan in 0.9% saline into the plantar region of the foot. The opposite foot

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injected with 0.9% saline was used as a base line. The standards indomethacin (10 mg kg^{-1}) and phenylbutazone (50 mg kg^{-1}) were used to compare activity. In Sprague Dawley male rats obtained from Charles River Lab. (Raleigh, NC) (242–260 g) the same test was repeated, but the drugs were given orally [PO] by intubation needles and the dosage was in the same range as in the mouse.

Protection against septic shock. CF_1 male mice (29–31 g) were administered lipopolysaccharides (LPS) *Salmonella abortus equi* [Lot #69F4003] at 10 mg kg^{-1}, I.P which has an LD_{100} within 48–52 h which was consistent with literature values.[6,7] Drugs were administered 2 h prior and 2 h post-injection of the LPS and then subsequently for every 24 h for the length of the animals' lives. Deaths were recorded every 12 h and continued for 96 h. Indomethacin (8 mg kg^{-1}) and pentoxyfylline (50 mg kg^{-1}) were used as standards.

Anti-pleurisy activity. Male Sprague Dawley rats (238–254 g) were administered drugs 8 mg kg^{-1} orally 1 h before and 3 h after injection of 0.05 ml of a 0.316% Evan's blue + carrageenan solution into the pleural cavity.[8] After 6 h, the rats were sacrificed by cervical dislocation; fluid was collected from the pleural cavity. The treated animals were compared to both control values and rats treated with the standards.

Local analgesic activity. CF_1 male mice (28.5–32 g) were administered agents at 8 mg/kg IP 20 min before 0.5 ml of 0.6% acetic acid was administered I.P.[9,10] After 5 min, the number of stretches were counted over the next 10 min. Indomethacin was used as a standard.

Hot plate tail flick screen. CF_1 male mice (29–32 g) were administered drugs at 8 mg kg^{-1}, IP prior to placement on a hot plate maintained at 100 °F. Time elapsed prior to tail raising was measured using a digital read-out connected to the hot plate.[11] Tail flick responses of CF_1 mice injected with morphine were used as the standard for this assay.

Collection of inflammation exudates for chemical mediator examination

In order to determine the effects of agents on the release of chemical mediators, i.e. cytokines or lymphokines, a number of biological fluids were collected from *in vivo* experiments. A modified sponge test[7,12] was performed in CF_1 male mice (28.5–31.4 g). A 1.0 $cm^2 \times 5$ mm thick [~30 mg] sponge saturated with 2.0 mg carrageenan was implanted subcutaneously in the abdominal wall. The mice were treated with agents at 8 mg kg^{-1}, IP 2 h prior to surgery and 2 h post surgery. Estimation of PMN migration to the site of inflammation was determined after collection of sponges 6 h post-implantation by preparing a 0.5% homogenate of the sponge in hexadecyltrimethylammonium bromide (HTAB).[13] Aliquots of the homogenate were used to determine PMN myeloperoxidase (MPO) activity using *o*-dianisidine dihydrochloride at 460 nm. Monocyte and/or macrophage migration to the site was determined by leaving sponges in place for 6–9 days. Homogenates of the sponge were prepared in HTAB; *N*-acetylglucosaminidase (NAG) lysosomal activity was determined by using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide. The hydrolysis product, *p*-nitrophenol, was determined at 400 nm.

In vivo *TNFα, Il-1 and Il-2 measurements.* CF_1 male mice (~30 g) were administered agents at 8 mg kg^{-1}, IP. After 3 h, LPS (*Salmonella abortus equi*) at 5 mg kg^{-1}, IP was administered. Blood was collected in non-heparinized tubes 15–120 min after LPS injection. Serum was obtained by centrifuging at 3500 RPM × 10 min. Serum tumor necrosis factor (TNFα) levels were determined in a bioassay using cultured L_{929} mouse cells[14,15] grown in Dulbecco's modified Eagle's medium (DMEM) + 10% fetal calf serum (FCS) + penicillin/streptomycin (P/S). Cytotoxicity was determined with 0.2% crystal violet in 20% MeOH; the number of living cells was determined at 580 nm using SOFTmax (Molecular Devices). The bioassay was quantitated with TNFα standards and confirmed with a mouse ELISA kit (Genzyme Corp., Cambridge, MA). Blood levels of Il-1 and Il-2 were determined similarly using mouse ELISA kits (Cytokine Research Products).

In vitro *TNFα and Il-1 measurements.* IC-21 mouse macrophages were maintained in RPMI-1640 + 10% FCS + P/S. After the cells had grown to confluency, *E. coli* LPS at 10 μg ml^{-1} was added to the medium. Agents were incubated at 1, 10 and 100 μM final concentration for 18 h. The medium (100 μl) was collected for TNFα determinations. Interleukin-1 (Il-1) release was

Table 1 The anti-inflammatory effects of metal amine carboxyboranes at 8 mg kg^{-1} day^{-1} in rodents

	Control (%)				
$N=6$ Compound	Anti-inflammation, CF_1 mice	Anti-inflammation, Sprague Dawley Rat	Writhing reflex, mice	Tail flick	Anti-pleurisy
1	41 ± 4[a]	55 ± 5[a]	17 ± 4[a]	143 ± 7[a]	
2	73 ± 5[a]		76 ± 5[a]	101 ± 6	
3	69 ± 5[a]		70 ± 3[a]	156 ± 6[a]	
4	82 ± 6		42 ± 4[a]	338 ± 8[a]	
5	74 ± 4[a]		36 ± 4[a]	599 ± 7[a]	
6	59 ± 5[a]	78 ± 7[a]	85 ± 4	184 ± 6[a]	40 ± 4[a]
7	49 ± 4[a]	70 ± 6[a]	66 ± 3[a]	150 ± 4[a]	96 ± 5
8	65 ± 6[a]	60 ± 7[a]	66 ± 4[a]	169 ± 6[a]	30 + 3[a]
9	77 ± 6[a]				
10	62 ± 4[a]				21 ± 4[a]
Phenylbutazone[b]	53 ± 4[a]				8 ± 5[a]
Indomethacin[c]	22 ± 3[a]	27 ± 3[a]	43 ± 4[a]		12 ± 3[a]
Morphine[d]				213 ± 5[a]	
1% CMC Control	100 ± 5	100 ± 6	100 ± 5	100 ± 6	100 ± 4

[a] Student's 't' test. [b] 50 mg kg^{-1}. [c] 10 mg kg^{-1}. [d] 1 mg kg^{-1}.

determined using P_{388} cells. The L_{929} bioassay was used to quantitate Il-1 levels.

Prostaglandin cyclooxygenase activity. Mouse macrophages (5×10^6 cells) were incubated with agents (10^{-4} to 10^{-8} M final concentration) and ^{3}H-arachidonic acid (100 Ci mol^{-1}) for 60 min at 37 °C in a CO_2 incubator. The reaction was terminated with 2N HCl, the mixture extracted twice with ether and the organic layer evaporated. The residue was dissolved in ethyl acetate and plated on TLC silica gel plates. These were eluted with chloroform/methanol/water/acetic acid (90:8:1:0.8).[16,17] The plates were developed in iodine vapor and scrapped according to the R_f values of standard prostaglandins and counted in a Packard scintillation beta counter corrected for quenching. IC_{50} values were calculated for each agent with a semi-log plot.

5'-Lipoxygenase activity. Mouse macrophages (5×10^6 cells) were incubated with agents at 10^{-4} to 10^{-8} M concentration. The cells were harvested by centrifugation and incubated in a phosphate buffer (pH 7.2), 0.6 mM $CaCl_2$, 1.0 mM $MgCl_2$, the calcium ionophore A 23187 and

Table 2 The protective effects of metal complexes of amine carboxyboranes against LPS-induced septic shock after 52 h

$N=6$	Alive (%)		
Compound	2 mg kg^{-1}	4 mg kg^{-1}	8 mg kg^{-1}
4	33 ± 4	83 ± 6	66 ± 6
5	0	17 ± 6	50 ± 5
6	100 ± 0	100 ± 0	100 ± 0
7	100 + 0	100 ± 0	100 ± 0
9	100 ± 0	100 ± 0	83 ± 4
10	67 ± 5	83 ± 5	83 ± 7
Indomethecin	0	50 ± 7	33 ± 4
Pentoxifylline (50 mg kg^{-1})			67 ± 6
Dexamethasone (1 mg kg^{-1})			67 ± 5

Table 3 The effects of metal complexes of boron derivatives on mouse J774 macrophage lysosomal enzyme activities after 60 min incubation

$N=6$	IC_{50} values × 10^{-6} M		
Compound	Cathepsin D pH 5.0	Acid phosphatase	Aryl sulfatase
1	4.57	0.82	5.43
2	1.92	2.00	1.33
3	2.28	1.42	1.60
4	2.85	2.09	1.95
5	3.39	2.44	2.75
6	2.49	2.48	1.20
7	3.83	2.92	1.95
8	2.38	3.08	2.38

^{3}H-arachidonic acid. After 20 min incubation at 37 °C, EtOAc/CH_2Cl_2 (2:3) supplemented with 12 mg arachidonic acid was added. The organic phase was extracted and dried. The residue was taken up in ethyl acetate and 100 ml was plated on silica gel TLC plates eluted with methylene dichloride/methanol/acetic acid/water (90:8:1:0.8). The plates were scrapped in the area that corresponds to 5HETE and counted.[18,19] IC_{50} values were calculated for each agent.

Lysosomal hydrolytic enzymatic activities. Acid phosphatase, aryl sulphatase, cathespin and trypsin proteolytic activities were measured utilizing mouse macrophages J774 (1×10^6 cells). Mouse macrophage J774 A.1 (American Type Culture BALB/C) tissue cultures were maintained in DMEM with 10% FCS and P/S. Agents were incubated from 10^{-8}–10^{-3} M for 60 min at 37 °C. Acid phosphatase activity was determined using 0.1 M β-glycerolphosphate in 0.1 M acetate buffer at pH 5.0. The reaction was stopped with 10% TCA and centrifuged at 3000 g × 6 min. The supernatant's inorganic phosphate was determined by the spectrophotometric method of Chen *et al.*[20] The net inorganic phosphate released in 30 min was corrected by subtracting the blank value at time zero. Aryl sulphatase activity was measured using 0.72 μmol of *p*-nitrocatechol sulfate as a substrate in 0.2 M acetate buffer, pH 5.0 for 30 min at 37 °C. The reaction was terminated with 4N NaOH. The formation of *p*-nitrocatechol in the supernatant was measured spectrophotometrically at 510 nm and corrected for blank values.[21] Cathepsin activity was determined using 2% azocasien as the substrate in 0.1 M acetate buffer, pH 5.0 for 30 min at 37 °C. The reaction was terminated with 10% TCA and centrifuged. The supernatant was assayed for acid-soluble peptide fragments at 366 nm and corrected for blank values.

Trypsin proteolytic activity was determined by the method of Schleuning and Fritz[22] using 2.0 ml of 0.1 M Tris buffer, pH 8.0 and 6 mM of *N*-benzoyl-L-arginine ethyl ester (BAEE) substrate. The hydrolysis of BAEE was determined after 30 min at 253 nm and blank values were subtracted. Elastase activity was determined by the method of Kleinerman *et al.*[23] using 2.9 ml of 0.2 M Tris-HCl buffer, pH 8.0, 2 units (200 μl) of porcine pancreatic elastase (Sigma, Type III) and 20 μl *N*-succinyl-L-alanyl-L-alanine-*p*-nitroanilide (Sigma, 100 mg in 5 ml of methyl-2-pyrrolidone). The cleaved product, *p*-nitroanilide, was determined at 410 nm after 3 min. Collagenase activity was determined by the method of Hu *et al.*[24] using 1 ml of 50 mM Tris + 5 mM $CaCl_2$ buffer, pH 7.6, 10 μg ^{3}H-collagen *N*-[propionate 2,3-^{3}H]propionylated (0.51 mCi mg^{-1}) and 10 μg collagenase (Sigma, Type II *Clostridium histolyticum*) incubated for 24 h at 37 °C. The reaction was stopped with 1 ml 50 mM EDTA. The tubes were then centrifuged at 10 000 g for 15 min. The supernatant was counted (Packard Tricarb liquid scintillation counter). Background radioactivity values were subtracted from each sample and corrected for quenching.

in vitro Determinations of agent toxicity

Intestinal mucosa cells. HCT-8 human small intestinal mucosa cells were grown to confluency in RPMI 1640 + 10% horse serum + sodium pyruvate + P/S.[25] Agents were incubated with

Table 4 The effects of metal complexes of boron derivatives on mouse hepatic lysosomal enzyme activities after 60 min incubation

$N=6$	IC_{50} values × 10^{-6} M			
Compound	Cathepsin D	Acid phosphatase	Aryl sulfatase	% Protection: Fenton reaction
1	6.19	4.00	4.34	2.2
2	6.69	3.56	5.15	21.9
3	7.65	3.58	2.83	24.2
4	6.84	5.60	2.57	2.1
5	8.17	4.14	2.33	8.5
6	5.47	6.99	7.73	1.6
7	7.80	4.25	5.89	15.2
8	4.58	7.66	3.05	0.0

Table 5 The effects of metal complexes of boron derivatives on mouse J774 macrophage proteolytic enzyme and prostaglandin cyclooxygenase activities after 60 min incubation

$N=6$	IC_{50} values $\times 10^{-6}$ M		
Compound	Trypsin BAEE	Elastase	Prostaglandin cyclooxygenase
1	2.25	3.07	15.59
2	2.27	7.26	2.41
3	2.21	6.35	2.07
4	2.37	3.92	2.34
5	1.81	4.11	2.08
6	2.10	5.09	1.62
7	1.38	9.77	2.09
8	2.38	5.93	1.92

plated cells at 10^{-8} to 10^{-4} M final concentration. The cells were washed, fixed in 95% MeOH, and incubated with 0.2% crystal violet. After the dye was washed from the cells, plates were read at 580 nm with a SOFTmax well counter program.

Enzymatic parameters. Enzymes leaking from HCT-8 cells into the supernatant were determined as a measure of agent toxicity. Lactate dehydrogenase activity was determined using pyruvate and the oxidation of NADH at 340 nm. Alkaline phosphatase activity was determined with β-glycerolphosphate in acetate buffer (pH 8.0). The inorganic phosphate released was measured in ammonium molybdate and ascorbic acid at 720 nm.[26] β-Galactosidase activity was determined with lactose in Tris-maleate buffer (pH 7.0). The glucose released was measured with Sigma Kit 520 at 450 nm. Leucine aminopeptidase activity was determined using L-leucine-*p*-nitroaniline in Tris-KCl buffer (pH 8.0). The *p*-nitroaniline released was measured at 405 nm.[27] cAMP levels were determined using a ^{125}I-RIA New England Nuclear kit. The cycotoxicity of the agent was correlated with its ability to cause release of these enzymes and cAMP if the agent damaged intestinal brush-borders and tight junctions on these cells.

RESULTS

The metal complexes and salts of amine carboxyboranes demonstrated good anti-inflammatory activity at 8 mg kg^{-1} in rodents (Table 1). Compounds **1**, **6** and **7** demonstrated greater than 40% reduction of induced edema in mice. Compounds **1** and **8** at 8 mg kg^{-1} were equally active in rats. Compounds **1**, **4** and **5** reduced the writhing reflex by more than 50% in mice; compounds **4** and **5** caused a three to six-fold protection against central pain induced by a hot plate in mice. Compounds **6**, **8** and **10** reduced pleural effusion in the thoracic cavity by at least 50%. Protection against endotoxic shock induced by LPS *Salmonella* at 52 h was afforded at all three doses of compounds **6** and **7** (100%) (Table 2). Compound **9** was 100% effective at 2 and 4 mg kg^{-1} day^{-1}; it was 83% effective at 8 mg kg^{-1} day^{-1}. Compound **4** was most effective at 4 mg kg^{-1} day^{-1} (83% protection). Similarly, compound **10** afforded 83% protection at 4 and 8 mg kg^{-1} day^{-1}. Sponge MPO activity at 6 hr was reduced 53% by **4**, 63% by **6**, 37% by **7**, 64% by **9**, and 65% by **10**. On days 6 and 9, compound **5** inhibited NAG activity from macrophages 22 and

Table 6 The effects of metal complexes of boron derivatives on proteolytic enzyme and prostaglandin cyclooxygenase activity after 60 min incubation

$N=6$	IC_{50} values $\times 10^{-6}$ M		
Compound	CF_1 Mouse hepatic trypsin	Porcine seminal vesicles P.g. Cyclooxygenase	*Clostridium histolyticum* commercial collagenase
1			
2	9.98	2.40	490
3	9.95	3.10	506
4	9.93	2.90	478
5	9.91	4.17	475
6	9.96	2.14	567
7	9.88	4.01	522
8	9.99	2.57	495

Table 7 The effects of metal complexes of boron derivatives on chemical mediator release

	in vivo		*in vitro*					
	Plasma levels in LPS-induced CF_1 mice treated with boron derivatives (8 mg kg^{-1})		TNFα and Il-1 release from LPS-induced macrophages incubated with boron derivatives					
	Control (%)		Control (%)					
$N=6$	TNFα		Il-1	Il-2	TNFα			Il-1
Compounds	90 min	180 min	180 min	90 min	10^{-6} M	10^{-5} M	10^{-4} M	10^{-5} M
4	145 ± 5[a]	25 ± 3[a]	—	21 ± 3[a]	212 ± 5[a]	70 ± 6[a]	43 ± 5[a]	56 ± 6[a]
5	197 ± 5[a]	—	—	35 ± 4[a]	64 ± 4[a]	58 ± 5[a]	50 ± 4[a]	103 ± 7
10	268 ± 7[a]	55 ± 4[a]	79 ± 5[a]	47 ± 3[a]	200 ± 9[a]	120 ± 5	106 ± 5	57 ± 5[a]
Control LSP	100 ± 4[b]	100 ± 5[c]	100 ± 4[d]	100 ± 4[e]	100 ± 5	100 ± 4	100 ± 4	100 ± 6

[a] Student's 't' test. [b] 0.316 ng ml^{-1}. [c] 0.789 ng ml^{-1}. [d] 277 pg ml^{-1}. [d] 0.157 O.D. unit μl^{-1}. [e]

64%, respectively. On these days, compound **10** reduced NAG activity 26 and 21%, respectively.

Compounds **1–8** resulted in IC_{50} values, i.e. the concentration required to inhibit 50% enzyme activity, in the range of 10^{-6} M or the hepatic and macrophage lysosomal enzyme activities, i.e. cathepsin D, acid phosphatase and aryl sulphatase (Tables 3 and 4). Hepatic proteolytic activities, e.g. trypsin and commercial collagenase, were also inhibited with IC_{50} values of 9.9×10^{-6} M and $4.75–5.67 \times 10^{-4}$ M, respectively. Macrophage trypsin and elastase activity were also inhibited by compounds **1–8** with IC_{50} values *ca.* 10^{-6} M (Tables 5 and 6). Commercial porcine seminal vesicles and macrophage prostaglandin cyclooxygenase activity was inhibited with IC_{50} values of $1.51–4.01 \times 10^{-6}$ M. TNFα levels were reduced after incubation of macrophages with boron derivatives **4** and **5** (Table 7). Blood levels of Il-2 after administration of **4**, **5** and **10** to CF_1 mice were significantly lowered whereas TNFα levels were elevated at 90 min (Table 7). These levels were then lowered after 2 h. IL-1 levels released from macrophages after *in vitro* incubation with drugs at 10^{-4} M were reduced by compounds **4** and **10**. This decrease exceeded 40%. *In vivo*, compound **10** lowered IL-1 levels by 21% 90 min after administration.

Human HCT ileum cells were incubated with the compounds at 10^{-4} M concentration (Table 8).

Table 8 *in vitro* HCT-8 toxicity of amine borane derivative

in vitro							
Cytotoxicity and enzyme levels in HCT-8 ileum mucosal cells incubated with 10^{-4} M boron derivatives							
Control (%)							
Cytotoxicity				Enzyme level			
$N=5$ Compounds	Crystal violet	Methylene Blue	LDH	Alkaline phosphatase	Leucine aminotransferase	α-Glucosidase	cAMP
4	120 ± 5	104 ± 6	85 ± 5	100 ± 6	104 ± 6	101 ± 6	98 ± 8
5	126 ± 6	119 ± 8	143 ± 6[a]	102 ± 5	130 ± 7	100 ± 7	69 ± 5[a]
10	38 ± 2[a]	79 ± 6[a]	32 ± 4[a]	99 ± 6	98 ± 5	103 ± 7	55 ± 6[a]
Control	100 ± 4	100 ± 7	100 ± 6	100 ± 7	100 ± 5	100 ± 7	100 ± 5

[a] Student's "t" test.

Only compound **10** was cytotoxic to these ileum mucosal cells at 10^{-4} M, but not at 10^{-6} M. Leakage of enzymes from intestinal mucosa villa, measured as lactic dehydrogenase, alkaline phosphatase, leucine aminopeptidase and α-glucosidase, was negative, cAMP release was also negative.

DISCUSSION

The metal complexes and salts of amine carboxyboranes demonstrated significant anti-inflammatory activity, but were not as active in these rodent screens as the simple amine carboxyboranes.[1] Their activities were more consistent with phenylbutazone and aspirin products rather than indomethacin or pentoxifylline standards. More importantly, these compounds were potent with regard to blocking endotoxic shock induced by *Salmonella* LPS affording 100% protection over 52 h with selected agents. Control animals demonstrated 84% deaths at this time. These agents reduced induced pleural effusions and blocked both local pain (writhing reflex) and central pain (tail-flick assay).The data suggest that the metal complexes of amine carboxyboranes are potent inhibitors of lysosomal enzyme release from macrophages invading the inflammation site. They also suggest that these agents are proteolytic enzyme inhibitors of cathepsin, trypsin and elastase which function in the spread of the inflammation. Activities of prostaglandin cyclooxygenase, which regulates the syntheses of PgH, PgF2a, PgE and 5'-lipoxygenase which regulates leukotrienes C4, D4, E4 and F4 which are inhibited by these metal complexes. Leukotrienes are capable of vasoconstriction, increased vascular cell wall permeability, mucosal secretions and neutrophil chemotaxis and influx, edema, release of lysosomal enzymes and generation of free oxygen radicals. Both the prostaglandins and leukotrienes are mediators of local inflammation. Thus these metal complexes can be described as dual inhibitors. The reduction of these mediators may be related directly to the excellent *in vivo* activity in the induced edema and septic shock syndromes. Reduction of TNFα and Il-1 release from invading white blood cells is important in reducing the inflammatory process, because Il-1 is as pyrogenic factor, stimulates synthesis of acute phase proteins, is chemotaxic to PMNs and causes release of Il-3 or colony stimulating factor.[25] TNFα can produce acute phase responses, i.e. a component of septic shock, and can stimulate the release of chemical mediators, i.e. Il-1, PAF and leukotrienes. The process brings about destruction of tissues, *e.g.* joint, muscles and synovial membranes. The metal complexes of amine carboxyboranes are potent anti-inflammatory agents which would be effective agents *in vivo* to block inflammatory responses.

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